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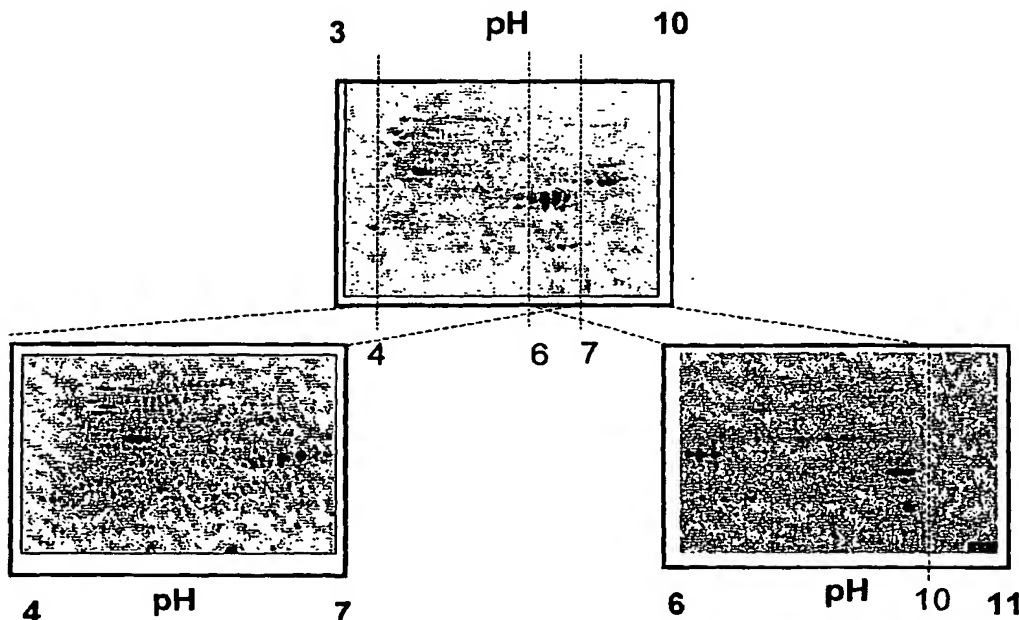
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[Continued on next page]

(54) Title: UNEXPECTED SURFACE PROTEINS IN NEISSERIA MENINGITIDIS



(57) Abstract: 217 proteins have, contrary to expectations, been found in the membrane of *Neisseria meningitidis*. Of these 217, 76 in particular evaded all algorithmic methods for predicting membrane localisation. Existing knowledge of protein trafficking pathways in meningococcus does not explain how or why these proteins are located in the bacterial membrane *e.g.* there is no apparent biochemical reason for a DNA helicase or a chromosomal replication initiator protein to be found in the membrane. These 217 proteins are provided as membrane proteins.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/095 C07K14/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLAASSEN I ET AL: "Production, characterization and control of a <i>Neisseria meningitidis</i> hexavalent class 1 outer membrane protein containing vesicle vaccine" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 14, no. 10, 1 July 1996 (1996-07-01), pages 1001-1008, XP004057632 ISSN: 0264-410X	1-3
Y	page 1002, left-hand column same citations -/--	4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	-& VAN DER LEY P ET AL: "Construction of Neisseria meningitidis strains carrying multiple chromosomal copies of the porA gene for use in the production of a multivalent outer membrane vesicle vaccine" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 13, no. 4, 1995, pages 401-407, XP004057740 ISSN: 0264-410X page 404, right-hand column, paragraph 2 - page 405, right-hand column, paragraph 1 -----	
X	WO 01/52885 A (PIZZA MARIAGRAZIA ;RAPPUOLIRINO (IT); CHIRON SPA (IT); GIULIANI M) 26 July 2001 (2001-07-26) page 2 - page 10 page 50 - page 52 -----	1-3
X	WO 00/25811 A (GORRINGE ANDREW RICHARD ;HUDSON MICHAEL JOHN (GB); MICROBIOLOGICAL) 11 May 2000 (2000-05-11) page 2 - page 6 -----	1,2
Y	NORAIS NATHALIE ET AL: "Combined automated PCR cloning, in vitro transcription/translation and two-dimensional electrophoresis for bacterial proteome analysis" PROTEOMICS, vol. 1, no. 11, November 2001 (2001-11), pages 1378-1389, XP009032238 ISSN: 1615-9853 page 1382, left-hand column, paragraph 1 - page 1383, left-hand column, paragraph 1; table 2 -----	4
Y	DATABASE UNIPROT 'Online! EBI; 1 October 2000 (2000-10-01), TETTELIN ET AL.: "Cell division ATP binding protein FtsE" XP002284894 Database accession no. Q9K1R3 the whole document ----- -/--	4

INTERNATIONAL SEARCH REPORT

International Application No

PC 03/06281

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GRIFANTINI R ET AL: "Previously unrecognized vaccine candidates against group B meningococcus identified by DNA microarrays" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 20, no. 9, September 2002 (2002-09), pages 914-921, XP002272872 ISSN: 1087-0156 page 917, right-hand column, paragraph 2 - page 918, left-hand column, paragraph 1; figure 2; tables 2,3</p>	
A	<p>PIZZA M ET AL: "IDENTIFICATION OF VACCINE CANDIDATES AGAINST SEROGROUP B MENINGOCOCCUS BY WHOLE-GENOME SEQUENCING" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 287, no. 5459, 10 March 2000 (2000-03-10), pages 1816-1820, XP000986271 ISSN: 0036-8075 page 1817 - page 1818; table 1</p>	
A	<p>TETTELIN H ET AL: "COMPLETE GENOME SEQUENCE OF NEISSERIA MENINGITIDIS SEROGROUP B STRAIN MC58" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 287, 2000, pages 1809-1815, XP000914963 ISSN: 0036-8075 the whole document</p>	
A	<p>JOLLEY KEITH A ET AL: "Immunization with recombinant Opc outer membrane protein from Neisseria meningitidis: Influence of sequence variation and levels of expression on the bactericidal immune response against meningococci" INFECTION AND IMMUNITY, vol. 69, no. 6, June 2001 (2001-06), pages 3809-3916, XP002284891 ISSN: 0019-9567 page 3810</p>	
A	<p>WRIGHT J CLAIRE ET AL: "Immunization with the recombinant PorB outer membrane protein induces a bactericidal immune response against Neisseria meningitidis" INFECTION AND IMMUNITY, vol. 70, no. 8, August 2002 (2002-08), pages 4028-4034, XP002284892 ISSN: 0019-9567 page 4029</p>	

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>POLLARD A J ET AL: "The meningococcus tamed?" ARCHIVES OF DISEASE IN CHILDHOOD. ENGLAND JUL 2002, vol. 87, no. 1, July 2002 (2002-07), pages 13-17, XP002284893 ISSN: 1468-2044 page 15; table 2</p>	
A	<p>WO 01/09350 A (DALEMANS WILFRIED L J ;SMITHKLINE BEECHAM BIOLOG (BE); THIRY GEORG) 8 February 2001 (2001-02-08) examples 3-5</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/06281

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 (all partly)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Inventions 1-158: claims 1-5 (all partly)

Inventions 1-158:

A composition comprising (a) outer-membrane vesicles (OMV's) prepared from a first strain of *Neisseria meningitidis* and (b) one or more proteins which are present in OMV's prepared from a second strain of *Neisseria meningitidis*, but which are not present in OMV's prepared from said first strain. Furthermore the specification of (b), the use of OMV's of genetically-modified strains of *Neisseria meningitidis* comprising proteins not present in OMV's prior to modification and furthermore the specification of the protein of (b) as set out in claims 4 or 5. Each of the inventions 1-158 specifically and respectively relates to one of the proteins set out in claims 4 or 5 e.g. for invention 1, NMB0007 etc... to invention 158, NMB2159.

Inventions 159-375: claims 6-8 (all partly)

Inventions 159-375:

A lipid bilayer including each and respectively a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO's: 1-217 or diverse variants, fragments or hybrids thereof. Furthermore the specification of the lipid bilayer and the said lipid bilayer which does not include some native membrane components. Each of the inventions 159-217 specifically and respectively relates to one of the proteins set out in SEQ ID NO's 1-217 e.g. for invention 159, SEQ ID NO: 1 etc... to invention 375, SEQ ID NO: 217.

Inventions 376-592: claims 10-13 (all partly)

Inventions 376-592:

A protein comprising each and respectively an amino acid sequence selected from the group consisting of SEQ ID NO's: 1-217 or diverse variants, fragments or hybrids thereof. Furthermore a nucleic acid encoding each of said proteins. Each of the inventions 376-592 specifically and respectively relates to one of the proteins set out in SEQ ID NO's 376-593 e.g. for invention 376, SEQ ID NO: 1 etc... to invention 593, SEQ ID NO: 217.

INTERNATIONAL SEARCH REPORT

International Application No
P B 03/06281

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0152885	A	26-07-2001	AU 2875401 A BR 0107679 A CA 2397508 A1 CN 1416352 T EP 1248647 A1 WO 0152885 A1 JP 2003520248 T MX PA02006962 A NZ 520466 A	31-07-2001 06-07-2004 26-07-2001 07-05-2003 16-10-2002 26-07-2001 02-07-2003 13-12-2002 26-09-2003
WO 0025811	A	11-05-2000	AT 242640 T AU 760858 B2 AU 1056900 A BR 9914946 A CA 2349331 A1 DE 69908805 D1 DE 69908805 T2 DK 1126874 T3 EP 1297844 A2 EP 1126874 A2 ES 2197688 T3 WO 0025811 A2 JP 2002528515 T PT 1126874 T US 2003215469 A1	15-06-2003 22-05-2003 22-05-2000 10-07-2001 11-05-2000 17-07-2003 19-05-2004 29-09-2003 02-04-2003 29-08-2001 01-01-2004 11-05-2000 03-09-2002 31-10-2003 20-11-2003
WO 0109350	A	08-02-2001	AU 770360 B2 AU 6833600 A BR 0012974 A CA 2380840 A1 CN 1377415 T CZ 20020403 A3 WO 0109350 A2 EP 1208214 A2 HU 0203056 A2 JP 2003506049 T MX PA02001205 A NO 20020506 A PL 353891 A1 TR 200200275 T2 TR 200202448 T2 ZA 200200824 A	19-02-2004 19-02-2001 07-05-2002 08-02-2001 30-10-2002 15-05-2002 08-02-2001 29-05-2002 28-12-2002 18-02-2003 02-07-2002 02-04-2002 15-12-2003 21-05-2002 21-01-2003 22-09-2003